

Cleaved Caspase 9 Protocol

- 1) Heat slides in 58 degrees for 1 hour
- 2) Xylene 5 min x 2
- 3) Rehydrate slides (100% alcohol x 3 min → 95% alcohol x 3 min → 70% alcohol x 3 min)
- 4) PBS wash 3 min
 - Antigen Retrieval
20 minutes in sodium citrate buffer pH 6 in steamer (97 degrees), then cool 30 min at room temp
Sodium Citrate buffer: 2.05 mL of 1 M sodium citrate, 450 uL of 1 M citric acid in 250 mL total volume (water that is microwaved for 1.5 minutes)
- 5) Wash PBS 3 min
- 6) 3% H₂O₂ in methanol 20 minutes (25 mL 30% H₂O₂ in 225 mL Methanol)
- 7) Wash PBS 3 min
- 8) Block slides in TNB (100 uL) 30 minutes
- 9) Primary antibody (Cleaved caspase 9 AB Cam 1:100 ab52298) diluted in TNB 100 uL/slide overnight at 4 degrees
- 10) Wash TBST 3 x 5 min each
- 11) Secondary antibody rabbit IgG biotinylated 1:400 in TNB, 1 hour Room temp
- 12) Wash TBST 3x 5 min
- 13) SA-HRP 1:100 in TNB, 100 uL per slide – 30 min room temp
- 14) Wash TBST 3x 5 min
- 15) Apply 100 uL Biotinyl Tyramide for 6 min (1:50 with 1x amplification diluent, can use 50-60uL if not enough stock)
- 16) Wash 3x 5min TBST
- 17) SA-HRP 1:100 in TNB, 100 uL per slide – 1 hour room temp
- 18) Wash 3x 5min TBST
 - DAB development (5 mL H₂O with 4 drops Dab, 2 drops buffer, 2 drops H₂O₂)
- 19) Fast Green Stain (FG for 1 minute → 1% acetic acid 30 sec → dip H₂O)
- 20) Dehydrate (2 min each of 70% alcohol → 95% alcohol → 100% alcohol → xylene)
- 21) Coverslip